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AF/1651

Attorney Docket # 3029-75RCE

Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Tomohiro TSUJI et al.
Serial No.: 09/992,221
Filed: November 06, 2001
For: Method for Classifying and Counting Nucleated Bone Marrow Cells

Examiner: J. P. Weber
Group Art: 1651

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May 21, 2004
(Date of Deposit)

Kent H. Cheng
Name of applicant, assignee or Registered Representative

Kent H Cheng
Signature

May 21, 2004
Date of Signature

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

SIR:

This is an appeal, pursuant to 37 C.F.R. §1.192(a) from the decision of the Examiner in the above-identified application, as set forth in the Final Office Action wherein the Examiner finally rejected Appellants' claims. The rejected claims are reproduced in the Appendix A attached hereto. A Notice of Appeal was filed on March 24, 2004. This Appeal Brief is being submitted in triplicate.

The fee of $330.00 for filing an Appeal Brief pursuant to 37 C.F.R. §1.17(f) is submitted herewith. Any additional fees or charges in connection with this application may be charged to our Patent and Trademark Office Deposit Account No. 03-2412.

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REAL PARTY IN INTEREST

The assignee, Sysmex Corporation is the real party of interest in the above-identified U.S. Patent Application.

RELATED APPEALS AND INTERFERENCES

There are no other appeals and/or interferences related to the above-identified application at the present time.

STATUS OF CLAIMS

Claims 1-11 have been rejected as being unpatentable under 35 U.S.C. 103 (a) over Inami et al. (U.S. 5,298, 426) in view of Kim et al. (U.S. 5,559,037), Hansen et al. (U.S. 4,284,412), Hoffman et al. (U.S. 4,492,752) and Bentley (1995) and further in view of Kim et al. (U.S. 5,516,695). Claims 1-11 are on appeal.

STATUS OF AMENDMENTS

There have been no Amendments filed subsequent to the Final Office Action.

SUMMARY OF THE INVENTION

Appellant's invention is directed to a method of classifying and counting leukocytic cells and erythroid cells in a bone marrow fluid. The method comprises:

(1) (a) mixing a sample of the bone marrow fluid with an erythrocyte lysing agent to lyse erythrocytes in the sample, thereby rendering leukocytic cells, erythroid cells and lipid particles in the sample suitable for staining, and

(b) staining the sample with a fluorescent dye for producing a difference in intensity of fluorescence among the leukocytic cells, the erythroid cells, and the lipid particles;

(2) introducing the resulting sample to a flow cytometer to detect at least one kind of scattered light and at least one kind of fluorescence;

(3) classifying the lipid particles, the leukocytic cells and the erythroid cells by the difference in the intensities of their fluorescence and their scattered light; and

(4) obtaining a count of the leukocytic cells and erythroid cells in the step of (3).

The foregoing is brought out in independent claim 1. The remaining claims 2-11 add or further define additional features of the method in accordance with the present invention. For example, the method of claim 2 further classifies erythroid cells into at least two erythroid cell groups according to the maturity of each of the erythroid cells, and obtains a count of cells in each of the erythroid cell groups by the difference in the intensities of the fluorescence and the scattered light from the at least two erythroid cell groups. Claim 11 specifically detects the side scattered light in combination with the detection of fluorescence intensity, to classify the lipid particles, the leukocytic cells and the erythroid cells in the bone marrow fluid.

As shown in Fig. 1, according to the method of the present invention, the nucleated bone marrow cells, lipid particles, and ghost cells are distributed to form their respective clusters. Examples 1 and 2 specifically teach the method of classifying leukocytic cells, erythroid cells (including three different groups of erythroid cells) and provide the specific ratios of these cells in the tested bone marrow fluid in Tables 1 and 2.

ISSUES

This appeal is focused on a single issue: whether claims 1-11 are patentable under 35 U.S.C. 103 over Inami et al. (U.S. 5,298, 426) in view of Kim et al. (U.S. 5,559,037), Hansen et al. (U.S. 4,284,412), Hoffman et al. (U.S. 4,492,752) and newly added Bentley (1995) and further in view of Kim et al. (U.S. 5,516,695). More specifically, the issue is: whether one of ordinary skill in the art would have modified a method of classifying leukocytic cells and lipid particles as taught by Bentley, by substituting fluorescence detection for absorption detection, and by substituting scattered light detection for aperture impedance

detection to thereby classify leukocytic cells, lipid particles, and erythroid cells, as claimed in the present invention.

GROUPING OF CLAIMS

The nature of the Examiner's error is essentially the same for all of the claims. However, because the somewhat different features recited in the pending claims make some of the claims separately patentable from each other, the claims are grouped as follows:

Group I -- claims 1, and 3-10 , which stand or fall together.

Group II -- claim 2, which stands alone.

Group III -- claim 11, which stands alone.

ARGUMENT

A. GROUP I (CLAIMS 1, 3-10)

The Examiner rejected claims 1-10 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent 5,298,426 of Inami et al. in view of U.S. Patent 5,559,037 of Kim et al., U.S. Patent 4,284,412 of Hansen et al., U.S. Patent 4,492,752 of Hoffman et al. and newly added Bentley et al., Amer. J. of Clinical Pathology 104 (1), 60-4 (1995) and further in view of U.S. Patent 5,516,695 of Kim et al.

In our previous response dated March 4, 2003, we stated that none of the references previously cited by the Examiner, i.e. Inami et al., Kim et al., Hansen et al., Hoffman et al. and Kim et al., alone or in combination, teaches a method of classifying the lipid particles, leukocyte cells, and erythroid cells of a nucleated bone marrow as claimed in the present invention.

The Examiner subsequently cited newly added Bentley reference in combination with all the previously cited references to reject claims 1-10 as being obvious under 35 U.S.C. 103(a).

Bentley discloses a method of separating leukocytes from fat particles by using a Cobas-Helios analyzer. In the method of Bentley, in order to obtain the scattergram absorbance and cell size are measured after staining the marrow sample with a dye.

In the present invention, Appellants stains marrow sample with a fluorescent dye and then detects intensities of fluorescence and scattered light.

In the Examiner's opinion, the absorbance based on stained cells of Bentley is comparable to the instantly used fluorescence; the cell size based on aperture impedance is comparable to the light scattering of the present invention. The Examiner also stated that the scattergram of Bentley is similar to that of the present invention because the same underlying parameters were being measured, cell type and cell size. Hence, the Examiner stated "Although Bentley et al. use different "observables" in the determination of TNC count than instant method, these are recognized in the art to be functional equivalents to those instantly used.

In response to our rebuttal argument dated October 29, 2003, the Examiner admitted that there were some differences between light scattering and aperture, but the distinctions were not great. The person of ordinary skill in the art was aware of limitations of the methods and can assign a margin of error to the measurement. Likewise, the Examiner stated that both fluorescence and absorption were well known spectroscopic techniques in detecting stained cells. The Examiner did not provide any reference supporting the assertion that fluorescence and absorption are equivalent to each other. The Examiner concluded that the selection among these methods was an arbitrary matter of experimental design.

Further, in response to our arguments as to the different results obtained by the method of Bentley and the present invention, the Examiner stated that it was not necessary for each of the references to disclose distinguishing erythroblastic cells. The Examiner also referred to the primary reference Inami et al., which identifies erythroblastic cells. Finally, the Examiner concluded that the analysis of the present invention that was beyond that of Bentley resulted from improved software, without any explanation or evidences supporting this assertion.

Appellants respectfully traverse.

1. None of the absorption method, impedance method, and combination thereof of Bentley is respectively the equivalent of the fluorescence method, scattered light method, and combination thereof of the present invention.

To determine whether one element is or is not an equivalent of another, the Federal Circuit has frequently applied the "way, function, and result" tripartite test. See e.g. *Dawn Equipment Co. v. Kentucky Farms Inc.,* 140 F.3d 1009, 1016 (Fed. Cir. 1998).

As to the absorption and fluorescence methods, the Examiner concluded that they are equivalents to each other merely because both are spectroscopic techniques, without providing any supportive evidences. Appellants respectfully traverse. There are a number of spectroscopic techniques, and many of them differ from each other in way, function, and result, and are thereby not equivalent to each other. Hence, Appellants believe that the Examiner failed to properly make a case of equivalence. As Appellants explained previously, these two methods are different from each other and are not interchangeable. For example, the absorbance method used by Bentley determines the degree of light absorbed by cells and compares it to the degree of light transmitted through cells by applying light of a certain wavelength to the cells. The fluorescence method determines the intensity of fluorescence radiated from the cells. Hence, the theoretical and physical bases of these two methods are different from each other.

More importantly, the fact that the result of the present invention as a whole is different from the Bentley reference clearly shows that the combination of fluorescence and scattered light methods is not an equivalent of the combination of absorption and the impedance methods of the prior art. The differences between the present invention and Bentley are clearly shown by, e.g. Fig. 2 of the present invention, and Figs. 1-2 of Bentley. As shown in these figures, the lipid particles, the erythorid cells, and the leukocytic cells are distributed to form the distinctive clusters in the present invention, while the erythoid cells cannot be distinguished from other cells in accordance with the Bentley method. The advantage of the present invention clearly results from the use of the combination of fluorescence and scattered light methods, rather than from the improved software, as asserted by the Examiner (without any supportive explanation), as shown by these figures. In other words, even if the present invention and Bentley use the same software (which they cannot), the final analysis results are not the same, because the scattergrams of the present invention and Bentley are different.

2. Even if the absorption and impedance methods were respectively equivalents to the fluorescence and scattered light methods, the mere existence of equivalency would have not render claims of the present invention obvious under 35 U.S.C. 103 (a).

The Federal Circuit has clearly held that the mere existence of equivalency cannot establish obviousness under 35 U.S.C. 103(a). See *Application of Flint*, 330 F.2d 363 (CCPA, 1964); see also I*n re Scott* 323 F.2d 1016 (CCPA, 1963). In the *Application of Flin* case, Judge Rich stated:

> The examiner and the board appear to hold that the mere existence of 'functional and mechanical equivalence' establishes 'obviousness.' We think this involves a nonsequitur. Expedients which are functionally equivalent to each other are not necessarily obvious in view of one another. The statutory mandate of 35 U.S.C. § 103 is that the 'claimed subject matter be unobvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains.'
>
> Assuming, arguendo, that the pin ejection system of appellant and the releasable spring arm taught by Pollack are 'functionally equivalent,' it does not follow that the former would be 'obvious' to one of ordinary skill in view of the latter. On the contrary, we see no suggestion in Pollack's leaf spring arm and stud construction to employ a spring-loaded pin directly in the pivot socket even though the solicitor characterizes such construction as 'a slightly different disposition of the pin member' which results in a combination which is 'merely the obvious equivalent of that of Pollack.' We therefore do not find obviousness.
>
> The defect which we find in the reasoning employed below to support the rejection here is not only that it ignores the express provision of the statute as stated in section 103 but that it also ignores the fact that it is advantageous to the public in the promotion of progress of the useful arts, the Constitutional objective of the patent law, to provide inducement for the invention of devices which are the functional equivalents of devices already known. It is not the object of the policy behind the patent system to encourage satisfaction with or commercialization only of the first device for performing a given function that happens to come along. And for those who may be interested in promoting competition in the interest of the consuming public, the greater the number of functionally equivalent devices which are encouraged onto the market by patent protection, the better off the consumer will be. Therefore the test is obviousness of the invention and not whether it serves the same purpose as previous inventions.

Therefore, the critical issue here is not whether there is any equivalence existing, but whether the prior art references have taught or suggested the desirability of the modification proposed by the Examiner. Moreover, as the Federal Circuit held: "the mere fact that the prior art may be modified in the manner proposed by the examiner does not make the modification obvious unless the prior art suggested the desirability of the modification." *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992).

In the present case, the prior art reference does not teach or suggest substituting the absorption method for fluorescence method, the impedance method for scattered light method. Neither does Bentley nor any other cited references suggest any desirability to make the substitutions proposed by the Examiner. Nor has the Examiner explained why the proposed modification of Bentley in view of any other cited references would have been desirable. Indeed, as we explained previously, the absorption method and impedance method of Bentley are substantially different from the fluorescence method and scattered light method of the present invention. In this light, it is evident that the Examiner has engaged in an impermissible hindsight reconstruction of the claimed invention using the appellants' claims as a template to selectively piece together the teachings of the prior art.

Based on the forgoing reasons, Appellants believe that claims 1, 3-10 are not obvious in view of the prior art references cited by the Examiner. The Final Rejection of the claims in Group I should be reversed.

B. GROUP II (CLAIMS 2)

Since claim 2 depends from claim 1, the reasons set forth above in connection with claim 1 equally apply to the patentability of claim 2. Moreover, claims 2 further recites a step of "classifying erythroid cells into at least two erythroid cell groups according to maturity of each of the erythroid cells". The prior art reference Bentley fails to teach this additional feature of the present invention. This fact further bolsters that the present invention in accordance with claim 2 is patentable in view of Bentley and the other cited references.

Therefore, the Final Rejection of claim 2 in Group II should also be reversed.

C. GROUP III (CLAIMS)

Claims 11 is similar to claim 1 except that the detection of the intensity of scattered light is limited to **side** scattered light. Hence, the reasons set forth above in connection with claim 1 also equally apply to the patentability of claim 11. As explained by the specification, the intensity of side-scattered light can reflect the intracellular information of the cells such as the nuclear form of cells well. This fact further support that the aperture impedance method of Bentley, which is used to determine the cell size, is not an equivalent of the

method of detecting the intensity of side scattered light, which is used to determine the intracellular information of the cells.

Therefore, claim 11 is not obvious over Bentley and the other cited references under 35 U.S.C. 103(a). The Final Rejection of claim 11 in Group III should be reversed.

CONCLUSION

For the foregoing reasons, it is respectfully submitted that Appellants' claims are not rendered obvious and are, therefore, patentable over the art of record, and the Examiner's rejections should be reversed.

Respectfully submitted,
COHEN, PONTANI, LIEBERMAN & PAVANE

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Dated: May 21, 2004

APPENDIX

1. A method of classifying and counting leukocytic cells and erythroid cells in a bone marrow fluid comprising leukocytic cells and erythroid cells and lipid particles comprising the steps of:

(1) (a) mixing a sample of the bone marrow fluid with an erythrocyte lysing agent to lyse erythrocytes in the sample, thereby rendering leukocytic cells, erythroid cells and lipid particles in the sample suitable for staining, and

(b) staining the sample with a fluorescent dye for producing a difference in intensity of fluorescence among the leukocytic cells, the erythroid cells, and the lipid particles;

(2) introducing the resulting sample to a flow cytometer to detect at least one kind of scattered light and at least one kind of fluorescence;

(3) classifying the lipid particles, the leukocytic cells and the erythroid cells by the difference in the intensities of their fluorescence and their scattered light; and

(4) obtaining a count of the leukocytic cells and erythroid cells in the step of (3).

2. The method according to claim 1, further comprising the steps of:

classifying erythroid cells into at least two erythroid cell groups according to maturity of each of the erythroid cells, and obtaining a count of cells in each of the erythroid cell groups by the difference in the intensities of the fluorescence and the scattered light from the at least two erythroid cell groups; and

calculating the ratio of the classified cells in each of the erythroid cell groups to the total erythroid cell count.

3. The method according to claim 1, further comprising the steps of:

classifying lymphocytes and monocytes included in the leukocytic cells and obtaining a lymphocyte count and a monocyte count; and

calculating a myeloid cell count by deducting the obtained lymphocyte count and the obtained monocyte count from the leukocytic cell count; and

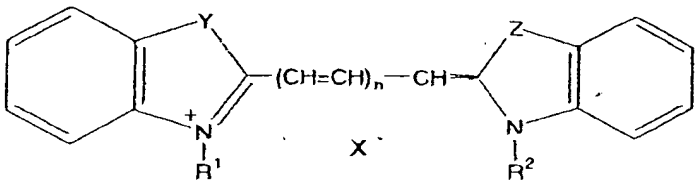
calculating the ratio of the erythroid cells to myeloid cells from the obtained myeloid cell

count and erythroid cell count.

4. The method according to claim 1, wherein the erythrocyte lysing agent is an aqueous solution having an osmotic pressure of 100 mOsm/kg or less and a pH of 2.0 to 5.0.

5. The method according to claim 1, wherein the fluorescent dye comprises one or more dyes selected from the group consisting of:

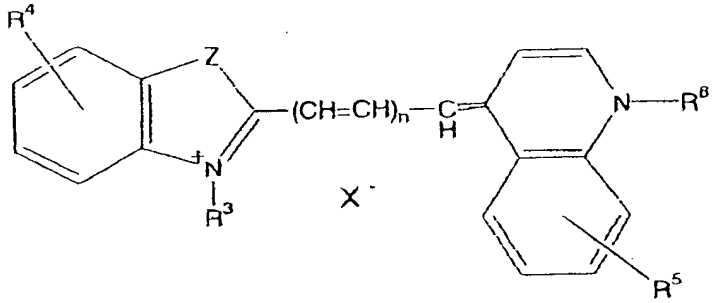
- compounds of formula (1)



(1)

wherein R^1 and R^2 are, the same or different, a hydrogen atom, or an alkyl or alkenyl group optionally substituted by a hydroxyl group; Y and X are, the same or different, a hetero atom or a carbon atom substituted by a lower alkyl group; n is 0, 1 or 2; and x^- is an anion,

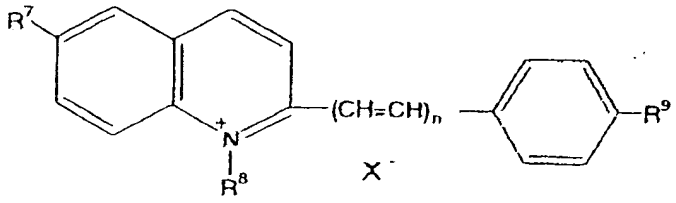
- compounds of formula (2)



(2)

wherein R^3 is a hydrogen atom or an alkyl group; R^4 and R^5 are, the same or different, hydrogen atom, a lower alkyl group or a lower alkoxy group; R^6 is a hydrogen atom, an acyl group or an alkyl group; Z is a hereto atom or a carbon atom substituted by a lower alkyl group; n is 0, 1 or 2; and x^- is an anion,

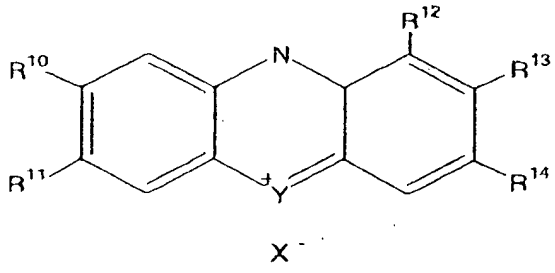
-compounds of formula (3)



(3)

wherein R^7 is a hydrogen atom or a dimethylamino group; R^8 is an alkyl group; R^9 is a hydrogen group or a dimethylamino group; n is 1 or 2; and x^- is an anion,

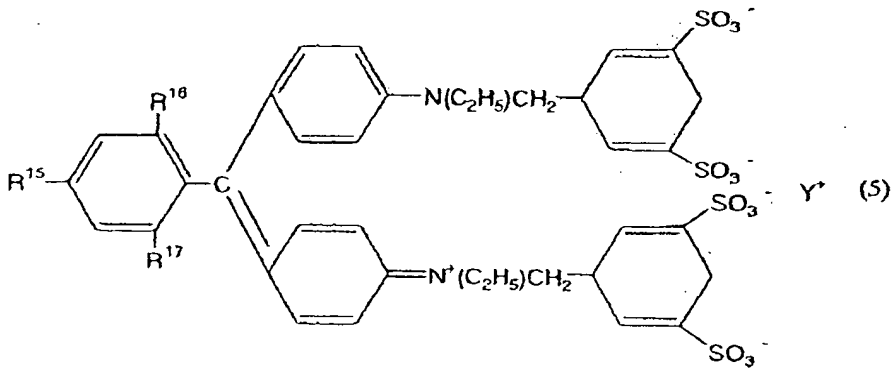
-compunds of formula (4)



(4)

wherein R^{10} is a hydrogen atom or an alkyl group; R^{11} is a dimethylamino group; R^{12} is a hydrogen atom or an amino group; R^{13} is a hydrogen atom, an alkyl group or an amino group; R^{14} is a hydrogen atom or a dimethylamino group; X^- is an anion; and Y is a hetero atom,

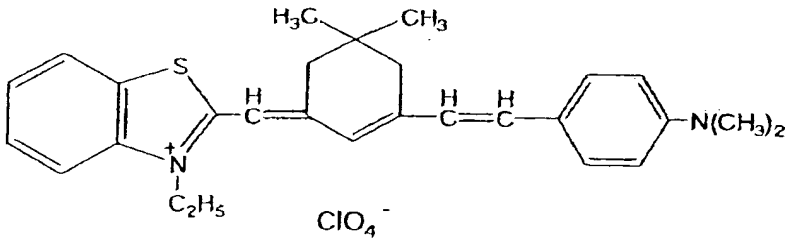
-compounds of formula (5)



(5)

wherein R^{15} is a hydrogen atom or a hydroxyl group; R^{16} is a hydrogen atom or a sulfonic group; R^{17} is a hydrogen atom or a sulfonic group; and Y is a cation,

-NK-2825:



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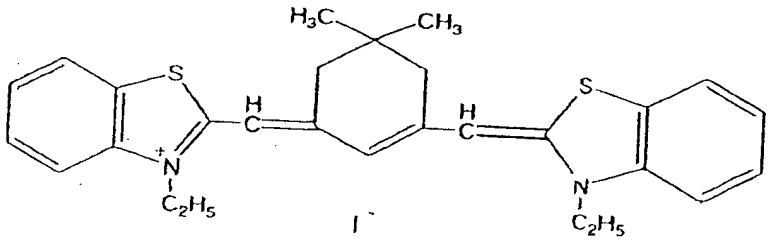

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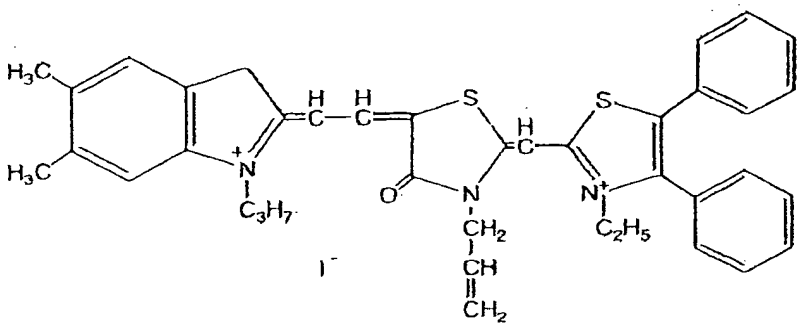

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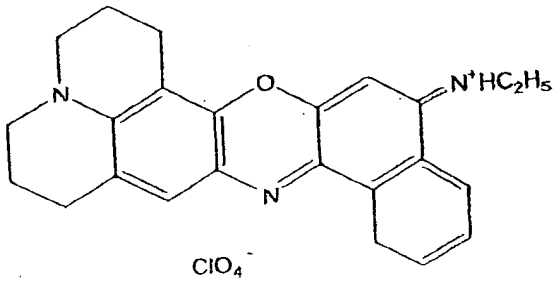
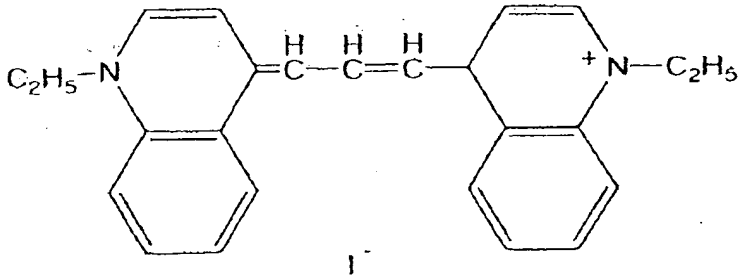


-Cryptocyanine:



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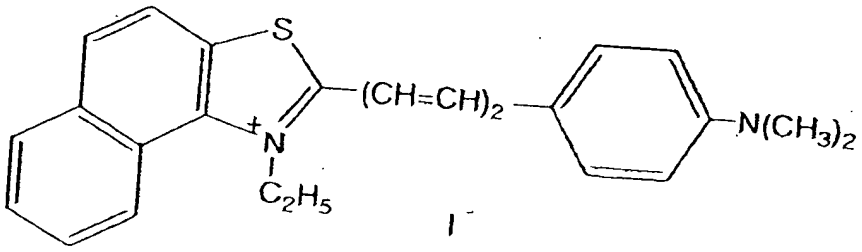

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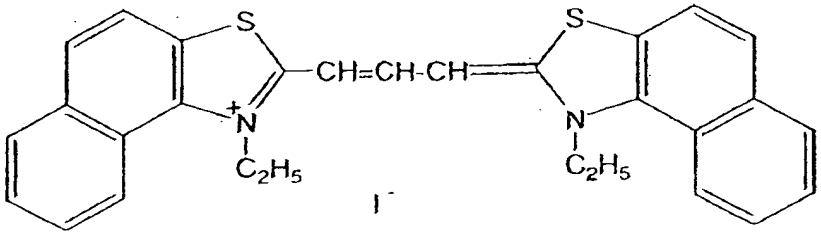

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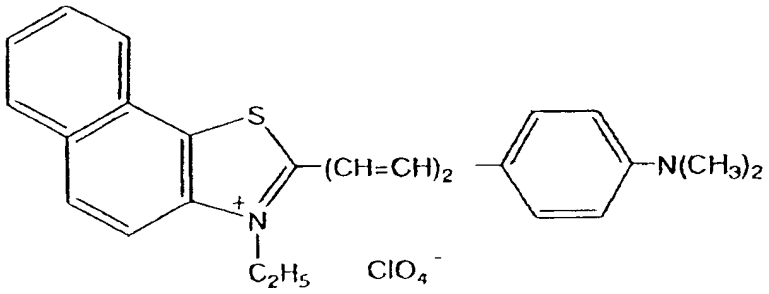

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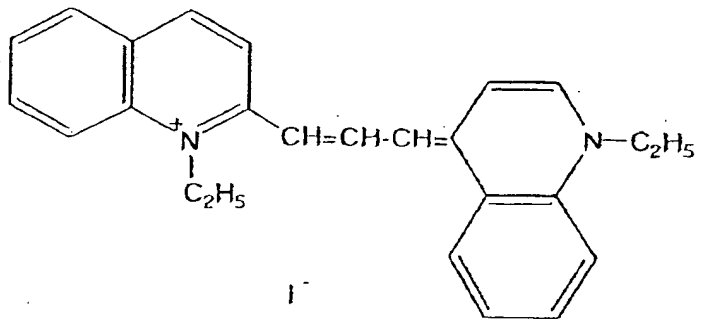

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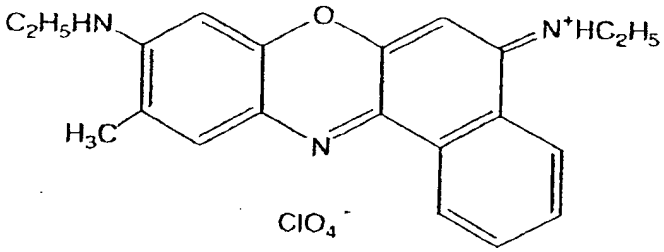

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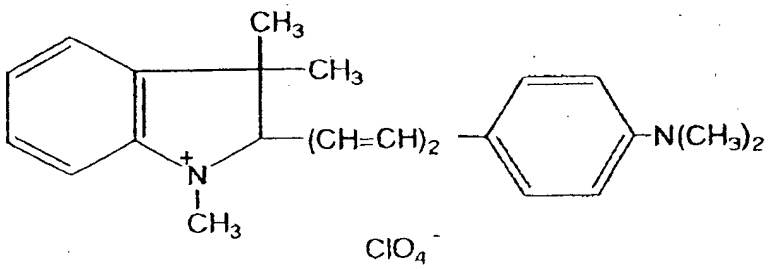

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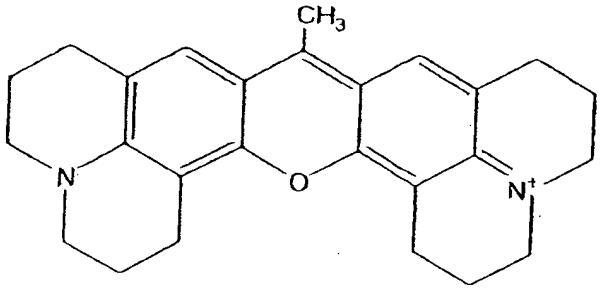
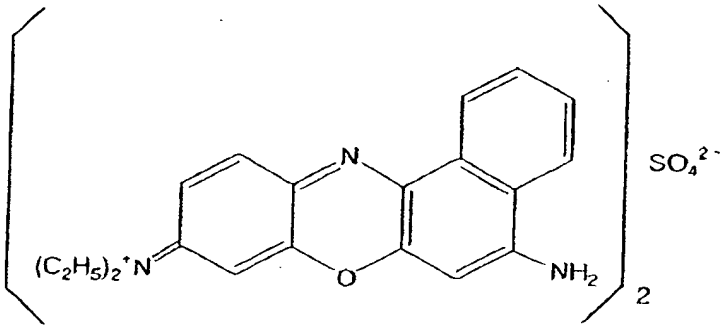
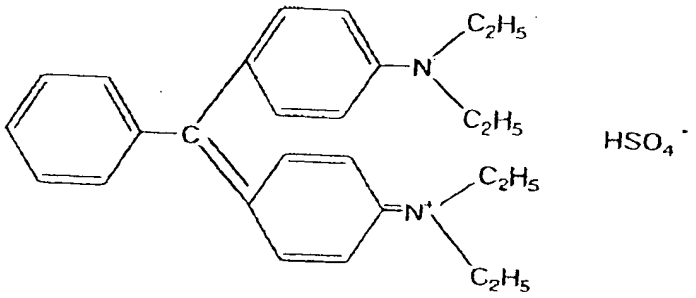


-Nile Blue A:



-Brilliant Green:



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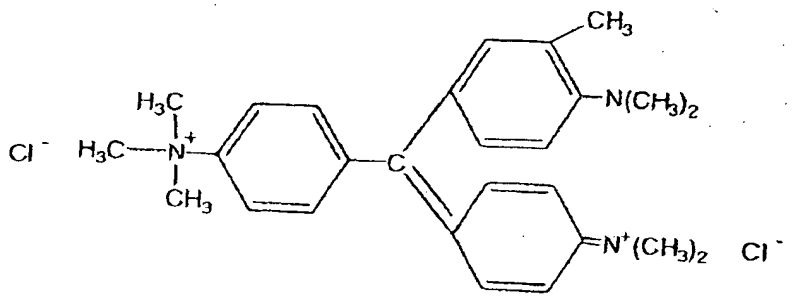
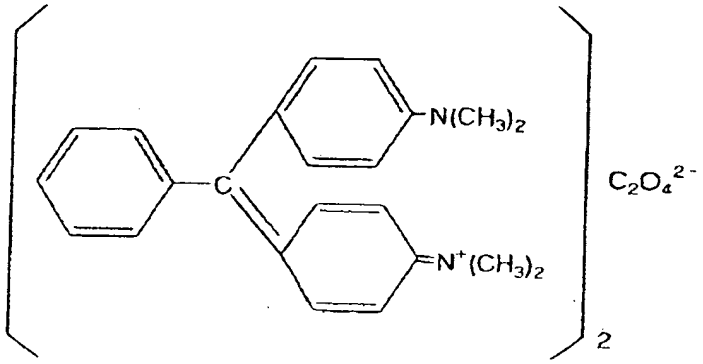


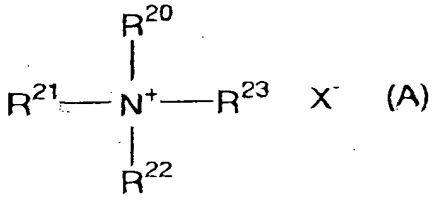
and

-Malachite green:



6. A method according to claim 1, wherein the erythrocyte lysing agent contains a surfactant, the surfactant comprises one or more surfactants selected from the group consisting of

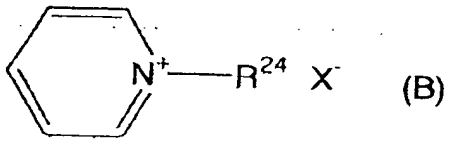
-compounds of formula (A)



wherein R^{10}, R^{21} and R^{22} are, the same or different, an hydrogen atom, a C_{1-8} alkyl group or a C_{6-8} aralkyl group; R^{23} is a C_{8-18} alkyl group, a C_{8-18} alkenyl group or a C_{6-18} alkenyl group or a C_{6-18} aralkyl

group; and x^- is an anion,

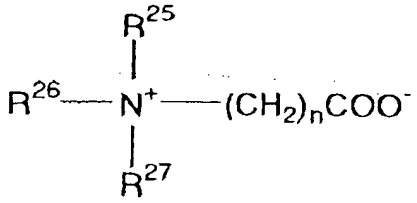
-compounds of formula (B)



(B)

wherein R^{24} is a C_{8-18} alkyl group; and X^- is an anion,

-compounds of formula (c)



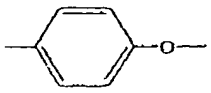
(C)

wherein R^{25} and R^{26} are, the same or different, a hydrogen atom, a C_{1-8} alkyl group, or a C_{6-8} aralkyl group; R^{27} is a C_{8-18} alkyl group, a C_{8-18} alkenyl group or a C_{6-18} aralkyl group; and n is 1 or 2,

-compounds of formula (D)

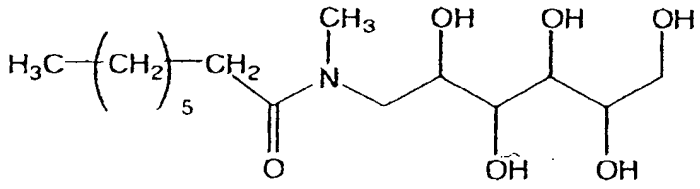
$$R^{28}-R^{29}-(CH_2CH_2O)_n-H \qquad (D)$$

wherein R^{28} is a C_{9-25} alkyl group, a C_{9-25} alkenyl group or a C_{9-25} alkynyl group; R^{29} is

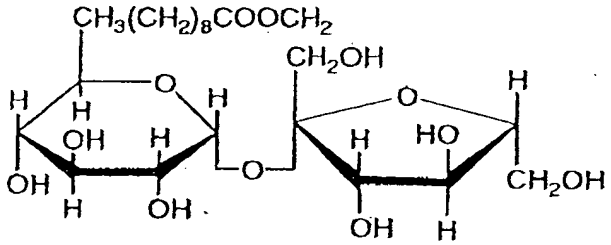


or $-COO-$; and n is an integer of 10 to 40,

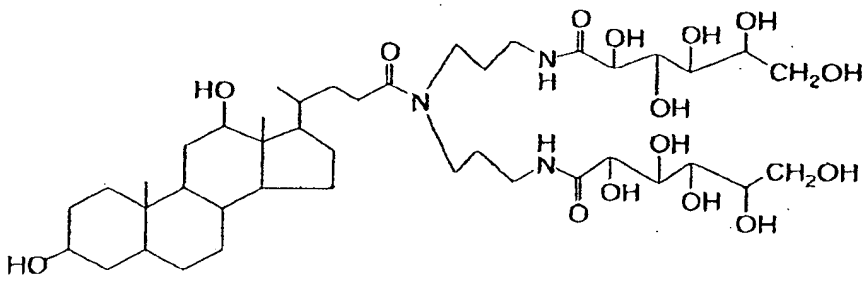
-MEGA-8:



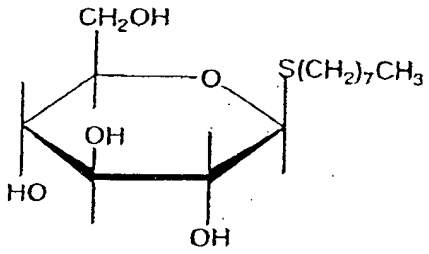
-sucrose monocaproate:



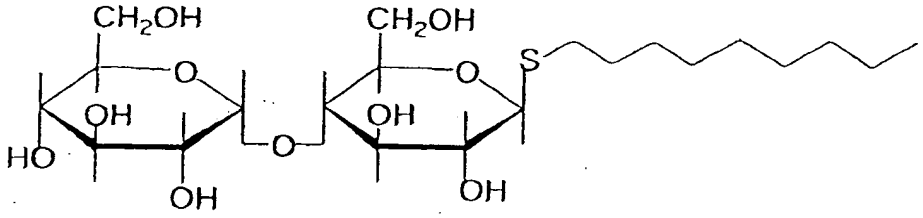
-Deoxy-BIGCHAP:



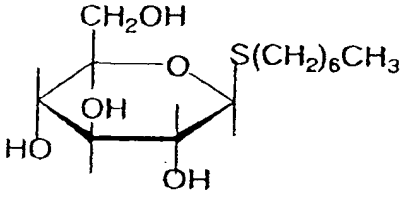
-*n*-octyl- β -D-thioglucoside:



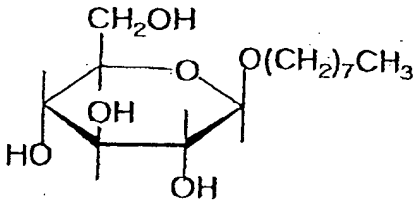
-n-nonyl- β -D-thiomaltoside:



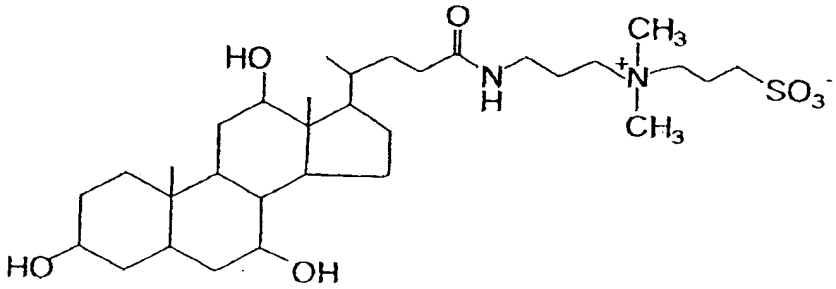
-n-heptyl- β -D-thioglucoside:



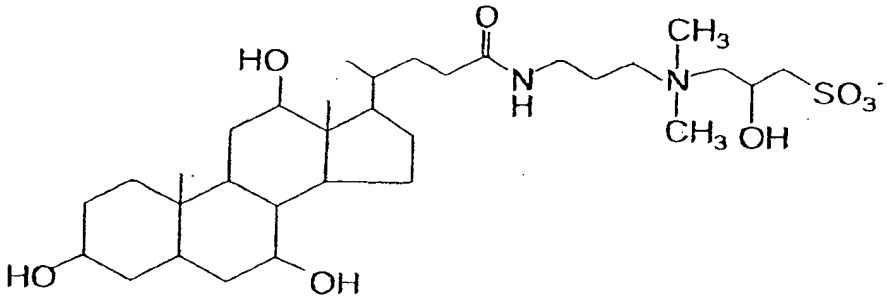
-n-octyl-D-oxyglucoside:



-CHAPS:



-CHAPSO:



7. The method according to claim 6, wherein the concentration of the surfactant is 10 to 10000 mg/L.

8. The method according to claim 1, wherein the detected scattered light is one or more kinds of scattered light selected from the group consisting of forward low-angle scattered light, forward high-angle scattered light and side scattered light.

9. The method according to claim 1, further comprising the step of:

calculating the ratio of the total count of leukocytic cells and erythroid cells to the count of erythroid or leukocytic cells.

10. The method according to claim 1, further comprising the step of:

calculating the ratio of the obtained leukocytic cell count to the obtained erythroid cell count.

11. A method of classifying and counting leukocytic cells and erythroid cells in a bone marrow fluid comprising leukocytic cells and erythroid cells and lipid particles comprising the steps of:

(1) (a) mixing a sample of the bone marrow fluid with an erythrocyte lysing agent to lyse erythrocytes in the sample, thereby rendering leukocytic cells, erythroid cells and lipid particles in the sample suitable for staining, and

(b) staining the sample with a fluorescent dye for producing a difference in intensity of fluorescence among the leukocytic cells, the erythroid cells, and the lipid particles;

(2) introducing the resulting sample to a flow cytometer to detect side scattered light and at least one kind of fluorescence;

(3) classifying the lipid particles, the leukocytic cells and the erythroid cells by the difference in the intensities of their fluorescence and their scattered light; and

(4) obtaining a count of the leukocytic cells and erythroid cells in the step of (3).